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Localization of possible functional domains in *sup*2 gene product of the yeast *Saccharomyces cerevisiae*

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Primary structures of yeast *sup*2 gene and polypeptide product coded by the gene are compared with the current nucleotide and amino acid sequence data base. The amino acid sequence of the *sup*2 product shows homology to elongation factors from different sources. Especially high homology is found in the regions, corresponding to conservative aminoacyl-tRNA- and GTP-binding domains, described in elongation factors and other proteins. The data obtained are discussed in relation to the functions of *sup*2 polypeptide product in protein synthesis.

Protein synthesis; GTP-binding site; aminoacyl-tRNA-binding site; Gene homology

1. INTRODUCTION

In the yeast *Saccharomyces cerevisiae* in addition to well known dominant suppressors coding for tRNA a new class of recessive suppressors has been described [1–4]. These suppressors designated *sup*1 and *sup*2 (similar to *sup*45 and *sup*35, respectively [4,5]) were found to be omnipotent, acting towards all three types of nonsense mutations (UAG, UGA and UAA), the suppression being mediated by an increase in the translational ambiguity [4,6,7]. These data indicate that *sup*1 and *sup*2 genes code for proteins controlling the accuracy of codon-anticodon interaction. Although the functional properties of these suppressors are well characterized [4,6,7] and both genes have recently been cloned [5,8–10], the opinion about the nature of their polypeptide products remains controversial. It seems that they combine the properties of ribosomal proteins [4,6,7] and protein factors [5,8] affecting different parameters of translation in yeast, in particular, the level of fidelity.

Here we present the results of a computer-assisted comparison of the *sup*2 gene polypeptide product with published sequences, allowing us to find considerable homology to elongation factors (EFs). Homologous regions include several domains in EFs, for which a functional role has been proposed earlier.

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2. MATERIALS AND METHODS

The cloning strategy for *sup*2 gene is described earlier [10]. Nucleotide sequence was determined following Sanger et al. [11]. The complete sequence of the *sup*2 gene and flanking regions will be published elsewhere. Primary structures were compared using the program GENEUS [12].

3. RESULTS

The search of nucleotide sequences homologous to the *sup*2 gene in the EMBL data bank (5789 se-

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quences) and further analysis on the amino acid level indicated the existence of homology of the *sup*2 gene product with yeast EF-1α and with analogous EFs from other species, mitochondria and chloroplasts. The highest level of homology to *sup*2 gene product was found for EF-1α from yeast [13,14] and brine shrimp, *Artemia salina* [15]. This homology spans the full length of EFs and permits an alignment of the *sup*2 gene product to either protein from EF-1α family.

A region of the *sup*2 gene product, homologous to EFs corresponds to a part of the open reading frame of the gene, starting from the third in-frame ATG codon to the termination codon. However, there are indications that this region may represent a functionally active protein. For example, plasmids, carrying the *sup*2 gene, in which initiation of translation on the first and second in-frame ATG codons is impaired due to deletion, retain the ability to complement a temperature-sensitive

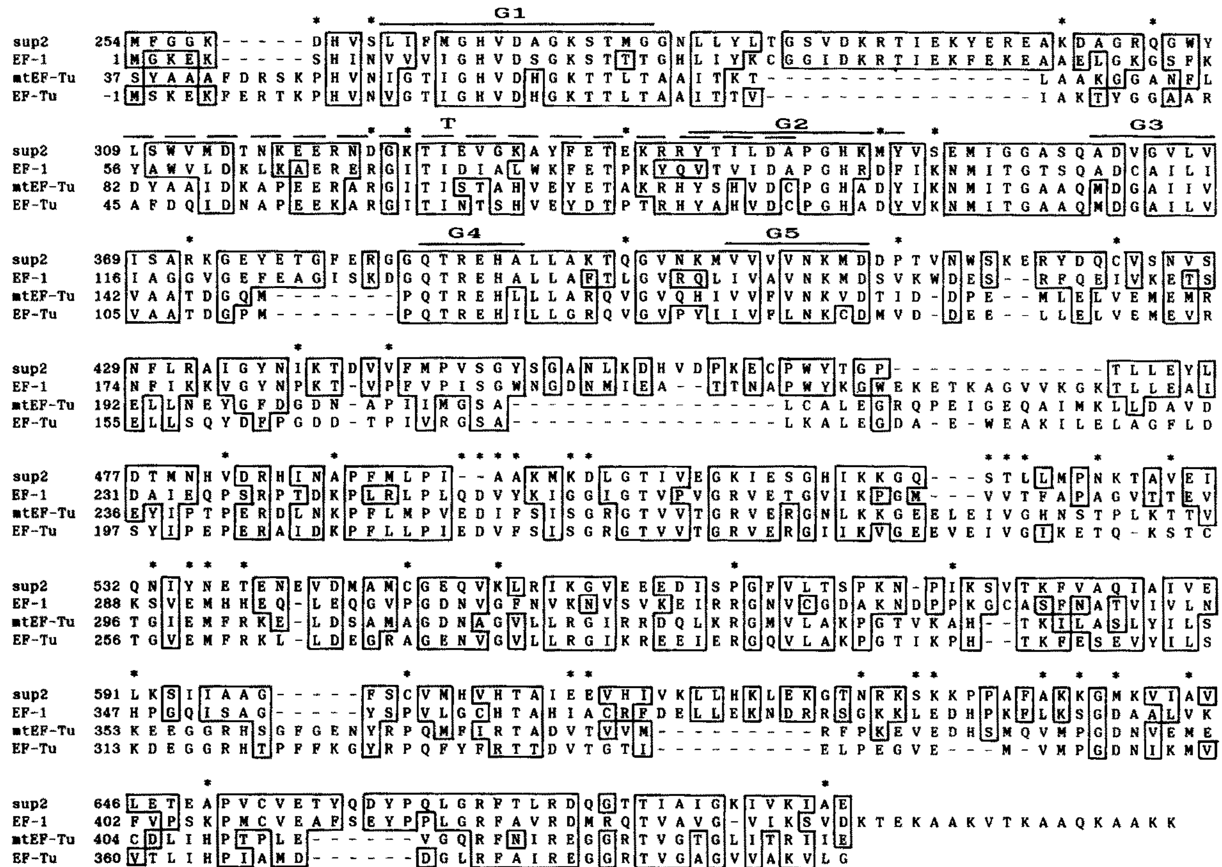

Fig.1. Comparison of the amino acid sequence of *sup*2 polypeptide product, yeast EF-1, mtEF-Tu and *E. coli* EF-TuA. The amino acid sequences of yeast *sup*2 polypeptide product, EF-1α [13], mtEF-Tu [16] and *E. coli* EF-TuA [17] are aligned to give maximal homology by introducing several gaps (–). The one-letter amino acid notation is used. The amino acid residue number 1 in the *sup*2 product is tentatively assigned to methionine at the first codon ATG in the open reading frame while that for EF-1α and mtEF-Tu to methionine at the initiator codon ATG and that for *E. coli* EF-TuA to serine, which is located at the N-terminal of the protein. The regions of exact homology and conservative substitutions between the *sup*2 product and either elongation factor are indicated by boxes. Conservative domains G_1–G_5 (involved in GTP-binding [19]) are indicated by solid lines, whereas region T (important for aminoacyl-tRNA binding) is shown by a dashed line. Positions, where EFs are homologous between themselves, but non-homologous to the *sup*2 product are marked by an asterisk (*). The following Dayhoff conservative categories [18] were used: C; S, T, P, A, G; N, D, E, Q; H, R, K; M, I, L, V; and F, Y, W.

mutation in the *sup*2 gene (Telckov, M., personal communication).

In fig.1 the *sup*2 gene product amino acid sequence, starting from the third methionine to the C-terminus (amino acids 254–685), is aligned to the sequences of yeast EF-1α [13], mitochondrial EF-Tu [16] and *E. coli* EF-TuA [17]. Comparison of the three latter sequences, belonging to evolutionary distant sources, reveals the most conserved regions of the EF-1α family. As seen from fig.1, most of them are present in the *sup*2 gene product sequence, although in some cases, amino acids conserved in the three proteins correspond to nonhomologous amino acids in the *sup*2 gene product (shown by asterisks). Considering conservative amino acid substitutions [18] as homologous and without counting the gaps, the sequence of the *sup*2 gene product shows 62% homology with yeast EF-1α and 43% with yeast mitochondrial EF-Tu. For comparison, homology between yeast cytosolic EF-1α and mitochondrial EF-Tu amounts to 55% [13].

From fig.1 one can see that the degree of homology is distributed unevenly along four sequences, the highest homology being in the N-terminus of EFs, which is where the conservative domains are located for which a functional role has been proposed [19–22]. Earlier the comparison of primary structures of several GTP-binding proteins, e.g. EFs, bacterial initiation factor IF-2α, *ras* proteins and bovine transducin, allowed deduction the structure of the GTP-binding site [19], including five conservative domains located sequentially. As shown in fig.1, a similar structural organisation is present in the *sup*2 gene product.

A functional role for another conservative domain in EFs was elucidated in the experiments on chemical modification and photooxidation of *E. coli* EF-Tu. A stretch of amino acids between 44 and 81 was shown to be involved in aminoacyl-tRNA binding [20–22]. A corresponding region homologous to this aminoacyl-tRNA binding domain is located between amino acids 308 and 345 in the *sup*2 product sequence (fig.1).

4. DISCUSSION

Upon alignment of the *sup*2 gene product with yeast EF-1α one can see rather high homology throughout almost the entire sequence. The predicted secondary structure of these proteins (α-helix, β-sheets, β-turns) as well as hydrophilicity distribution are very similar (not shown). This could mean that these two proteins may have similar tertiary structure and interact with common ligands. A significant homology found between a polypeptide product of the *sup*2 gene and aminoacyl-tRNA and GTP-binding domains in EFs of different origin may indicate that the amino acid sequences shown in fig.1 (G_1, G_2, G_3, G_4, G_5 and T) are specialized for performing the same functions in the *sup*2 gene product (GTP-binding, GTP-hydrolysis and aminoacyl-tRNA recognition).

These data together with a previous observation on the participation of the *sup*2 gene in the control of translational fidelity [4,6,7] allow us to suggest that a polypeptide product of the gene may perform GTP-dependent proofreading of codon-anticodon interaction in the ribosome acceptor site. The presence of structures homologous to aminoacyl-tRNA binding domain indicates that the *sup*2 gene product may directly participate in the process of aminoacyl-tRNA recognition.

It is important to note that participation in the control of translational fidelity is already proven for one of the proteins homologous to the *sup*2 gene product. In vitro studies of mutationally altered *E. coli* EF-Tu, namely EF-Tu Ar reveal that it increases the errors at both the proofreading and the initial aminoacyl-tRNA selection steps [23]. This mutation together with mutation inactivating the product of *tufB*, another gene for EF-Tu, suppresses all three types of nonsense mutations [24].

Despite structural similarity on the polypeptide level, EF-1α cannot functionally substitute the *sup*2 product since earlier [4] a number of conditionally lethal mutants of *sup*2 were isolated. These data indicate that the *sup*2 protein is indispensible for viability of the yeast cell. Another characteristic distinguishes the yeast EF-1α and the *sup*2 gene product, namely the codon usage. EF-1α is one of the most abundant proteins in yeast and the codon usage in its gene is highly biased in good agreement with the results of Bennetzen and Hall [25]. In contrast, the *sup*2 gene does not show a high level of codon bias (not shown) suggesting that it does not belong to the highly expressed gene group.

Although a part of the *sup2* gene product described in this paper possesses a high structural homology to EFs and in particular to yeast EF-1α and bacterial EF-Tu, the functional role of the *sup2* gene product in protein synthesis seems to be different and remains to be established.

REFERENCES

[1] Inge-Vechtomov, S.G. (1964) Vestn. LGU (USSR) 9, 112–117.

[2] Inge-Vechtomov, S.G. and Andrianova, V.M. (1970) Genetika (USSR) 6, 103–115.

[3] Hawthorne, D.C. and Leupold, U. (1974) Curr. Topics Microbiol. Immunol. 64, 1–47.

[4] Surguchov, A.P., Smirnov, V.N., Ter-Avanesyan, M.D. and Inge-Vechtomov, S.G. (1984) in: Physicochemical Biology Review (Skulachev, V.P. ed.) vol.4, pp.147–205, Soviet Scientific Reviews, Moscow.

[5] Himmelfarb, H.J., Maicas, E. and Friesen, J.D. (1985) Mol. Cell. Biol. 5, 816–822.

[6] Surguchov, A.P., Berestetskaya, Yu.V., Fominykch, E.S., Pospelova, E.M., Smirnov, V.N., Ter-Avanesyan, M.D. and Inge-Vechtomov, S.G. (1980) FEBS Lett. 111, 175–178.

[7] Eustice, D.C., Wakem, L.P., Wilhelm, J.M. and Sherman, F. (1986) J. Mol. Biol. 188, 207–244.

[8] Breining, P. and Piepersberg, W. (1986) Nucleic Acids Res. 14, 5187–5197.

[9] Breining, P., Surguchov, A.P. and Piepersberg, W. (1984) Curr. Genet. 8, 467–470.

[10] Telckov, M.V., Surguchov, A.P., Dagckesamanskaya, A.R. and Ter-Avanesyan, M.D. (1986) Genetika (Russian) 22, 17–25.

[11] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.

[12] Harr, R., Fallman, P., Haggstrom, M., Wahlstrom, L. and Gustafsson, P. (1986) Nucleic Acids Res. 14, 273–284.

[13] Nagata, S., Nagashima, K., Tsunetsugu-Yokota, Y., Fujimara, K., Miyazaki, M. and Kaziro, Y. (1984) EMBO J. 3, 1825–1830.

[14] Shirmaier, F. and Philippsen, P. (1984) EMBO J. 3, 3311–3315.

[15] Von Hemmert, F.J., Amons, R., Pluijms, W.J.M., Van Ormondt, H. and Moller, W. (1984) EMBO J. 3, 1109–1113.

[16] Nagata, S., Tsunetsugu-Yokota, Y., Naito, A. and Kaziro, Y. (1983) Proc. Natl. Acad. Sci. USA 80, 6192–6196.

[17] Yokota, T., Sugisaki, H., Takanami, M. and Kaziro, Y. (1980) Gene 12, 25–31.

[18] Dayhoff, M.O., Schwartz, R.M. and Oroutt, B.C. (1978) in: Atlas of Protein Sequence and Structure 5, Suppl.3, 345–352.

[19] Kohno, K., Vchida, T., Ohkubo, H., Nakanishi, S., Nakashi, T., Fuku, T., Ohtsuka, E., Ikehara, M. and Okada, Y. (1986) Proc. Natl. Acad. Sci. USA 83, 4978–4982.

[20] Laursen, R.A., L'Italien, J.J., Nugarkatti, S. and Miller, D.L. (1981) J. Biol. Chem. 256, 8102–8109.

[21] Arai, K., Kawakita, M., Nakamura, S., Ishikava, I. and Kaziro, Y. (1974) J. Biochem. (Tokyo) 76, 523–534.

[22] Nakamura, S. and Kaziro, Y. (1983) J. Biochem. (Tokyo) 90, 1117–1124.

[23] Tapio, S. and Kurland, C.G. (1986) Mol. Gen. Genet. 205, 186–188.

[24] Vijgenboom, E., Vink, T., Kraal, B. and Bosch, L. (1985) EMBO J. 4, 1049–1052.

[25] Bennetzen, J.L. and Hall, B.D. (1982) J. Biol. Chem. 257, 3026–3031.